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Experimental work performed during this program demonstrated that amino acids (phenylalanine and 3-hydroxytyrosine) could be reversibly, quantitatively and selectively detected on evanescent fiber optic sensors using immobilized acyclic polyether hosts supported on their outside surface. This investigation suggested that optical detection of the subject molecular recognition event may occur in the absence of a distinct absorbance change and be a probable consequence of changes in refractive index or dielectric properties at the interfacial region. This implied that the optical molecular recognition associated with interaction between the

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Abstract continued

evanescent wave and host/guest chemistry at a fiber optic waveguide can be extended to include the probing of more subtle optical changes at the fiber optic waveguide/host interfacial region.

Report #0001AC on ONR-SBIR Phase I Contract #N00014-89-C-0250

entitled

ADVANCED BIOSENSORS FOR AMINO ACID DETECTION

Final Report Period October 1, 1989 - March 31, 1990

INTRODUCTION

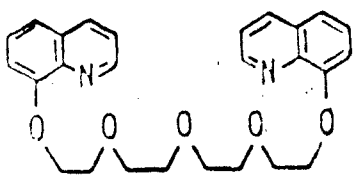
This Phase I program was directed towards determining the feasibility of an advanced biosensor technology compatible for the real-time optical detection of amino acids typified by phenylalanine, dopa (3-hydroxytyrosine), tryptophan and related compounds. Work performed unequivocally demonstrated feasibility for the originally proposed concept, with the general approach being found applicable to any host/guest molecular recognition event resulting in a detectable optical change originating from interaction with an evanescent wave within a fiber optic waveguide.

The strategy pursued involved amino acid detection by monitoring optical changes induced within immobilized chromagenic acyclic polyether host molecules to which the amino acid became associated. It was anticipated that the amino acid would become incorporated within a chromagenic polyether cavity via hydrogen bonding with the nitrogen atoms present, resulting in an optical change induced at an attached azo-dye chromaphore by withdrawing electron density from the nitrogen atom located in the polyether backbone cavity. The chromagenic polyether host was immobilized within a cellulose acetate film co-deposited onto the outside surface of the fiber optic sensor. High sensitivity for the detection of optical changes induced by the presence of amino acids was addressed by the use of synchronous sample-and-hold electronic circuitry.

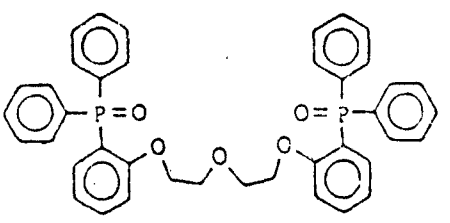
The approach was based upon the use of multifunctional host molecules which provided both a hydrophobic cavity suitable for initially binding the amino acid and a chromaphoric group whose optical properties might be perturbed by the resulting host/guest molecular interaction. The desired characteristics sought in this program by host molecules for facilitating the optical detection of amino acids included:

- Their ability to conveniently reversibly bind an amino acid guest molecule when immobilized onto a fiber optic substrate.
- That the resulting host/guest molecular association would induce some detectable optical change.
- The host and host/guest molecular species being insoluble in aqueous solution, with the amino acid guest being conveniently partitioned from the aqueous phase via a suitable semi-hydrophobic cavity within the host.

The ability to reversibly detect amino acids in aqueous solution was based upon the use of acyclic polyether cavities, as shown by I and II below incorporated into molecular hosts for reversible amino acid binding.



I



II

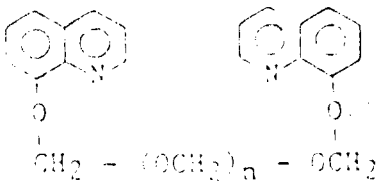
We were attracted to molecular hosts incorporating the above cavities because of:

- the relative synthetic ease with which molecular hosts containing these binding cavities may be prepared.[1]
- their compatibility towards tailoring host molecular substrates by varying either the polyether backbone or endgroups.

- their ability to incorporate into the polyether backbone a chromaphore whose optical characteristics may be perturbed upon binding with an organic ammonium cation.[2]
- previously demonstrated and relatively rapid reversible binding kinetics towards amines and amino acids as a consequence of their flexible polyether backbone.[1]

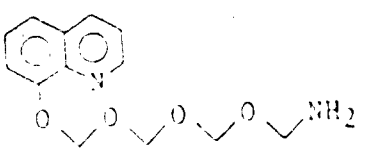
Selection of molecular hosts based upon (I) for amino acid detection was prompted by previous work which had shown that inclusion of quinoline terminal groups onto acyclic polyether backbones could result in hosts possessing good stability for complexation with organic ammonium cations.[3] Subsequent work had shown that the quinoline containing acyclic polyether (I) possessed high selectivity for binding, transport and release of amines and amino acids across an aqueous electrolyte/$CHCl_3$ liquid membrane/aqueous electrolyte cell[4] when (I) was present dissolved in the center chloroform layer. Here it was shown that transport of phentermine ($C_6H_5CH_2C(CH_3)_2NH_2$) or norephedrine ($C_6H_5CH(OH)CH(CH_3)NH_2$) by (I) occurs 170 and 62 times as rapidly, respectively, through a chloroform compared to either K^+ or Na^+. Furthermore, comparison[4] of transport rates among organic ammonium cations showed selectivities dependent upon ammonium cation hydrophobicities. Here transport rates were found to decrease in the order phentermine > phenethylamine > norephedrine > tyramine > dopamine. The last two organic amines (tyramine and dopamine) have incorporated into their aromatic rings hydroxy groups which decrease cation hydrophobicity, thus resulting in a weakening of binding to the acyclic polyether host. Thus, quinoline containing acyclic polyethers possess intrinsic selectivity not only for discrimination between inorganic cations and organic ammonium cations but also within groups of organic ammonium cations.

It has been found that factors important in the binding of organic ammonium cations by (I) include:[5] i) the presence of terminal quinoline groups which act as strong hydrogen binding sites for guest ammonium cations, and ii) their possessing a flexible acyclic polyether backbone permitting dynamic conformational changes to occur during the binding and releasing of ammonium cations. In addition, acyclic polyethers containing terminal endgroups such as (I) were considered to be attractive initial molecular hosts for amino acid binding since the cavity size, its hydrophobicity and the nature of the terminal endgroups could be varied with relative synthetic ease.[3] The thought here was that such modifications could in the future permit acyclic polyether tailoring for achieving high selectivity towards a given amino acid. Manipulation of the molecular host (I) might include, for example, adjusting the chain length (III), synthesizing unsymmetrical open-chain polyethers such as (IV), or enhancing rigidity within the polyether backbone by incorporation of aromatic groups (V) as shown below:

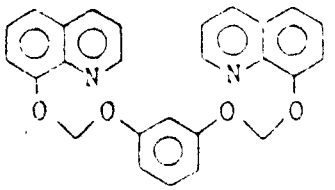


n = 0-6

III



IV

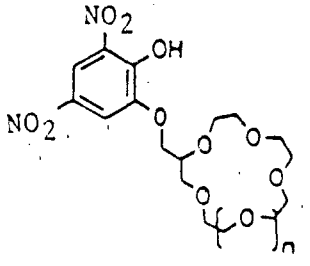


V

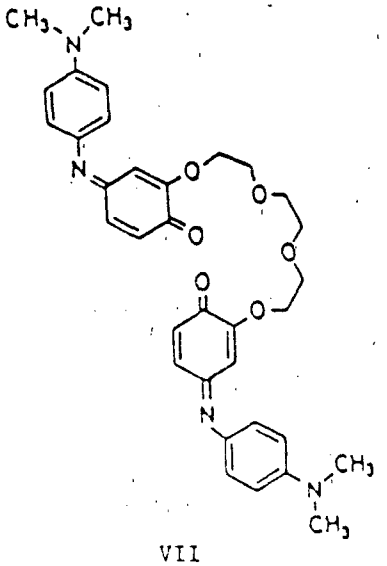
The related molecular host cavity (II) possessing phosphine oxide end-groups, has high formation constants for hydrogen bond formation[6] which, when incorporated into an appropriate molecular structure, we expected would provide a binding site for organic ammonium cations. This has in fact been previously demonstrated[7] when (II) was dissolved in $CHCl_3$, and found effective for extracting phenylethylamine ($C_6H_5CH_2CH_2NH_2$) and dℓ-phenylglycine ethyl ester ($C_6H_6(NH_2)COOCH_2CH_3$) from 0.2N aqueous perchloric acid solutions. In this case, some extraction selectivity was observed in that neither methylamine, ethylamine, propylamine or n-butylamine were extracted from aqueous solution.

In addition to molecular recognition and selectivity, a host molecular substrate should ideally also exhibit reversibility to the analyte of interest. Acyclic polyethers in general have been shown to exhibit more rapid reversible kinetics for complexation than crown ethers.[1] This property has been attributed to the flexible nature of the acyclic polyether backbone. Thus, as previously discussed, the ability of acyclic polyethers to bind an amine, transport it across a $CHCl_3$ liquid membrane followed by release into an aqueous phase[4,8] suggested to us that incorporation of a chromaphore to the acyclic polyether backbone might form the basis for amino acid optical detection.

Recent work has shown that chromaphoric groups can easily be incorporated into crown ethers to give ion selective colorimetric reagents.[9] For example, when a 1,2-dichloroethane solution of (VI) was brought into contact with an aqueous solution containing Na^+ species, the organic phase was found to turn from yellow (λmax = 273nm) to violet (λmax = 430nm).[10] Similar wavelength shifts have also been reported for acyclic polyethers such as (VII).[9a]

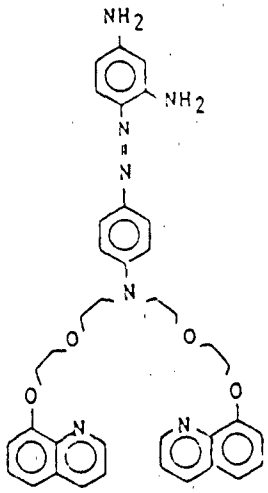


VI (n=2)

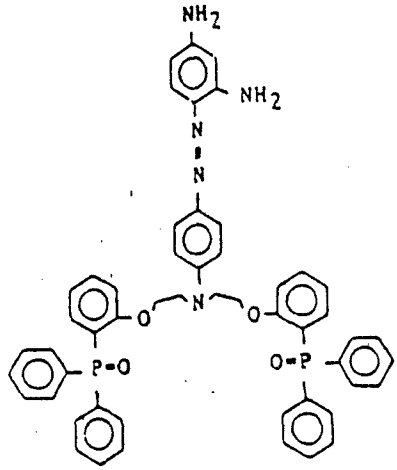


VII

This general strategy was applied during Phase I by replacing the central oxygen atom of molecular hosts (I) and (II) with nitrogen, introduced as part of an azo-dye chromaphore as shown in (VIII) and (IX).



VIII



IX

It was therefore anticipated in this Phase I program that incorporation of an amino acid into the above chromagenic acyclic polyethers via hydrogen bonding with proximate nitrogen atoms within the cavity, could result in inducing a detectable optical change associated with the attached azo-dye chromaphore, via a withdrawal of electron density from the nitrogen atom located in the polyether backbone.

In principle, azo-dyes possess a number of attractive features as chromaphoric indicator groups for amino acid binding including:

- a high extinction coefficient allowing facile detection of small quantities of amines and amino acids bound to the chromagenic acyclic polyether host.
- ease of synthesis and incorporation into the acyclic polyether host by use of well-established azo-coupling techniques.
- the fact that azo-dyes can be synthesized covering a wide spectral range, thus facilitating simultaneous multi-analyte amino acid detection.

The anticipated association between the acyclic polyether chromaphore and an amine or amino acid guest to be optically detected is schematically shown in Figure 1.

During this Phase I program chromagenic acyclic polyether hosts were immobilized onto the surface of fiber optic waveguides within a codeposited cellulose acetate film. The fiber optic waveguide (Figure 2) consisted of an optically transparent inner core surrounded by an outer core of lower refractive index (n_2). Removal of the fiber optic cladding permitted interaction of an evanescent wave with a medium external to the core such as the immobilized chromagenic host. Here interaction by the evanescent wave might occur to a depth (dp) of ~1000-2000Å.

Consequently when the exposed fiber optic is coated with a material whose interaction with the evanescent wave might be modified upon analyte binding, then a change in transmitted light through the fiber is induced, permitting

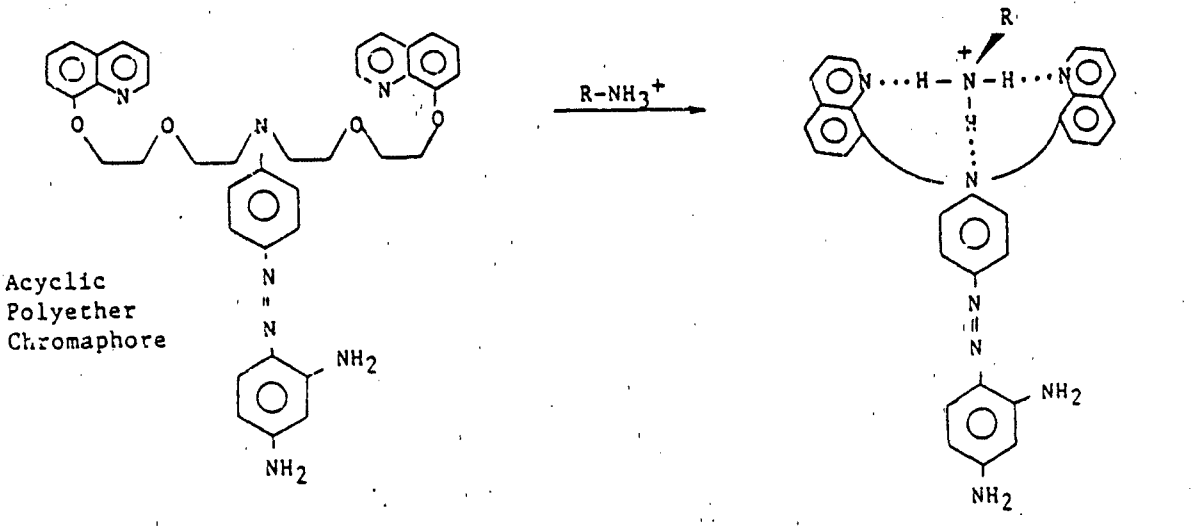


Figure 1. Detection of amines or amino acids ($R-NH_3^+$) by inducing optical changes within an azo-dye group attached to an acyclic polyether backbone following incorporation of the amine or amino acid guest into the cavity of the chromagenic acyclic polyether host.

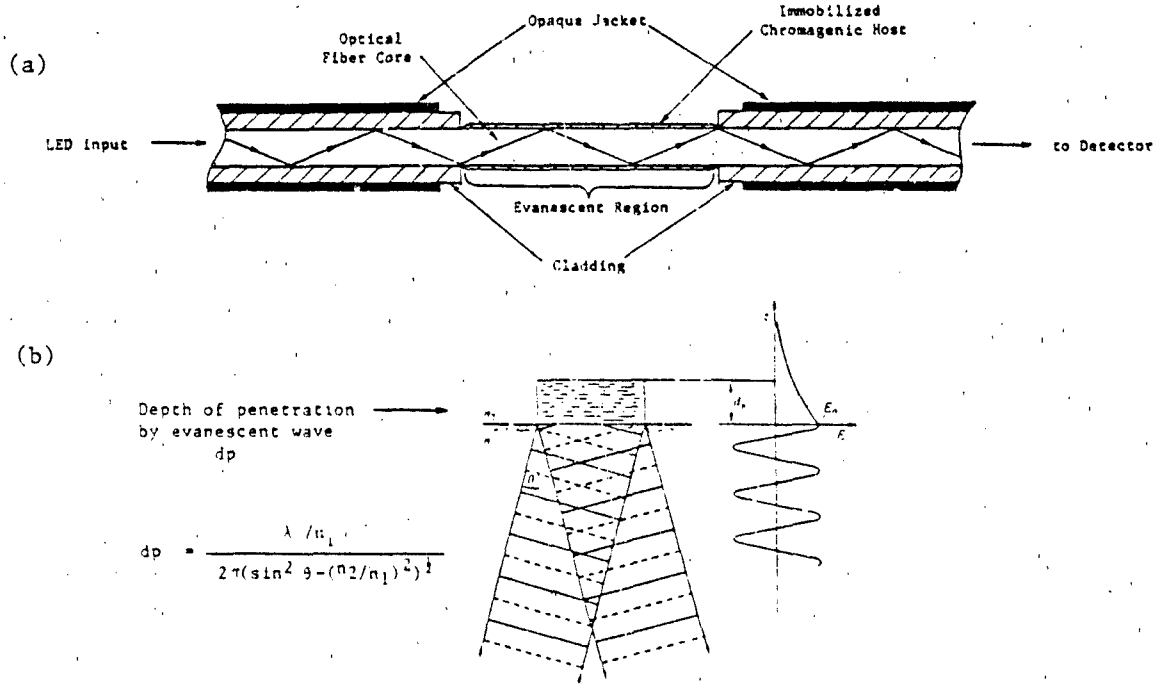


Figure 2. Schematic configuration for fiber optical detector (a) and generation of the evanescent wave at an interface between two optical media (b).

optical detection of the analyte. When transmitted light through an optical waveguide strikes the interface between two transparent media, going from a medium of greater refractive index to one of lower refractive index, total internal reflection will occur when the angle of reflection θ is larger than the optical angle θ_c as given by:[11]

$$\theta_c = \sin^{-1} n_2/n_1 \qquad (1)$$

In the above case, the evanescent wave will penetrate a distance dp into the medium with a lower refractive index (i.e. n_2). Although there will be no net flow of energy into the acyclic polyether chromaphore medium of lower refractive index, there will be an evanescent, nonpropagating field into this medium whose electric field amplitude E will be largest (i.e. E_o) at the interfacial region decaying exponentially with distance Z from the surface. E will be given by

$$E = E_o \cdot \exp(-Z/dp) \qquad (2)$$

The depth of penetration (dp) being defined as the distance required for the electric field amplitude to fall to exp(-1) of its value at the surface and is given by (Figure 2):

$$dp = \frac{\lambda/n_1}{2\pi(\sin^2\theta - (n_2/n_1)^2)^{\frac{1}{2}}} \qquad (3)$$

Generally, the penetration depth will depend on n_2 and n_1, the wavelength λ and the angle of incidence. Further specificity for the analyte being detected and its concentration might be achieved when the chromaphore exhibits a characteristic absorbance.

Experimental observations found in this Phase I program towards demonstrating the viability of the above discussed technical strategy for optically detecting amino acids using chromagenic acyclic polyether hosts immobilized in cellulose acetate films on the surface of fiber optic waveguides will now be discussed.

RESULTS AND DISCUSSION

Experimental work performed during Phase I of this program was directed towards demonstrating the feasibility of the previously discussed chromagenic host compounds, when immobilized onto fiber optic waveguides, for achieving the reversible optical detection of amino acids.

Specific technical objectives addressed during performance of this program included:

- Initially synthesizing chromagenic acyclic polyether hosts (VIII) and (IX).
- Evaluating changes in optical characteristics between 300 and 900nm for (VIII) and (IX) when immobilized within cellulose acetate membranes supported on glass slides prior to and after exposure to amino acids of interest.
- Evaluating the performance of chromagenic polyether hosts incorporated into cellulose acetate films coated on the outside of an unclad optical fiber waveguide.
- Determining the optical response achieved from the proposed detector when exposed to candidate amino acid solutions.

A. Syntheses of Chromagenic Polyether Host Molecules.

The objective here was to prepare the acyclic polyether chromagenic hosts (VIII) and (IX) for later immobilization onto the surface of a fiber optic waveguide for achieving the reversible detection of phenylalanine, dopa (d-hydroxytyrosine) and tryptophan.

The specifics as to how each of these chromagenic hosts were synthesized will now be discussed.

i) Syntheses of acyclic polyether chromaphore containing quinoline endgroups (VIII).

Initial syntheses performed used p-nitroaniline and 2-chloroethylether in the first step. Here 11ml (.112 moles) of 2-chloroethylether in 50ml DMF was added to 6.907g (0.05 moles) of p-nitroaniline also in DMF followed by refluxing overnight. However, subsequent gas chromatographic (GC) analysis of the reaction mixture using a Porapak Q column at 180°C showed essentially the same amount of 2-chloroethylether as in the starting mixture. Thus, no reaction was promoted. Since resonance effects are important in aromatic amines and since p-nitroaniline is an extremely weak nucleophile, lack of reactivity at this stage was, we interpreted, a consequence of the low basicity associated with the amine group which made attack at the C-Cl bond unlikely.

This suggested that use of an electron donating group para to the amine might help promote the desired reaction. We selected 4-aminoacetanilide where the acetamide group located para to the amino group was expected to donate electron density to the latter, thereby promoting the desired reaction. Furthermore, the acetyl part of the protecting group was available for convenient later removal via acid hydrolysis.

As an additional aid in promoting the desired reaction, we chose to add a base catalyst to the initial reaction mixture. Here ethyldiisopropylamine was chosen to promote alkylation of the amine group. Ethyldiisopropylamine is a strong sterically hindered base which we expected would preclude it alkylation by 2-chloroethylether. Thus in amine alkylation, it could serve as a proton acceptor without becoming alkylated itself. Specific details of the synthetic scheme will now be discussed and are summarized in Figure 3.

a) Condensation of 4-aminoacetanilide with 2-chloroethylether.

The first step involved addition of 7.50g of 4-aminoacetanilide in DMF to 11ml of 2-chloroethylether and 28ml of ethyldiisopropylamine during stirring. The mixture was then heated to 100°C for 12 hours followed by cooling and DMF removed under vacuum using a rotary evaporator, leaving a thick viscous liquid. This liquid was then dissolved into ~5ml of petroleum ether and separated on a neutral activated alumina column (Brockmann I, 150 mesh). A reddish-brown layer was collected and the petroleum ether evaporated to give a 33% yield (6.69g) of (X).

b) Addition of quinoline terminal endgroups.

Compound (X) (6.69g) in DMF was now added dropwise to a solution of 8-hydroxyquinoline (5.37g) in 30ml of DMF which in addition contained K_2CO_3 (5.1g). The mixture was then heated to 100°C overnight before cooling and filtering. DMF was removed using a vacuum rotary evaporator. Reaction products were separated on an alumina column as discussed above to give a reddish solid (compound XI) in 41% yield (4.39g).

c) Removal of acetyl and introduction of chromagenic group.

Compound (XI) (4.88g) was now dissolved in a 50/50 mixture of concentrated HCl/H_2O followed by heating to 50°C for removing the protecting acetyl group from the para-amino group to give (XII). The solution was then cooled to 0°C using an ice bath. Cold aqueous sodium nitrite (0.7g) was slowly added to the amine/HCl mixture. To this mixture 1,2-phenylenediamine (1.8g) in 0.2N HCl was now added with vigorous stirring. Aqueous sodium acetate was added until compound (VIII), the desired chromagenic host, precipitated. The precipitate was then filtered, neutralized with 20-40% aqueous NaOH and extracted into $CHCl_3$. Chloroform was evaporated and (VIII) purified using an alumina column and light petroleum ether as the elutant as discussed before. The yield for this final step was 52%, giving an overall yield of ~7% (2.6g). Elemental chemical analysis of the prepared compound closely agreed with that expected for (VIII). (Expected $C_{38}H_{39}H_7O_4$, found $C_{37.6}H_{39.2}H_{7.12}O_{3.8}$, Galbraith.)

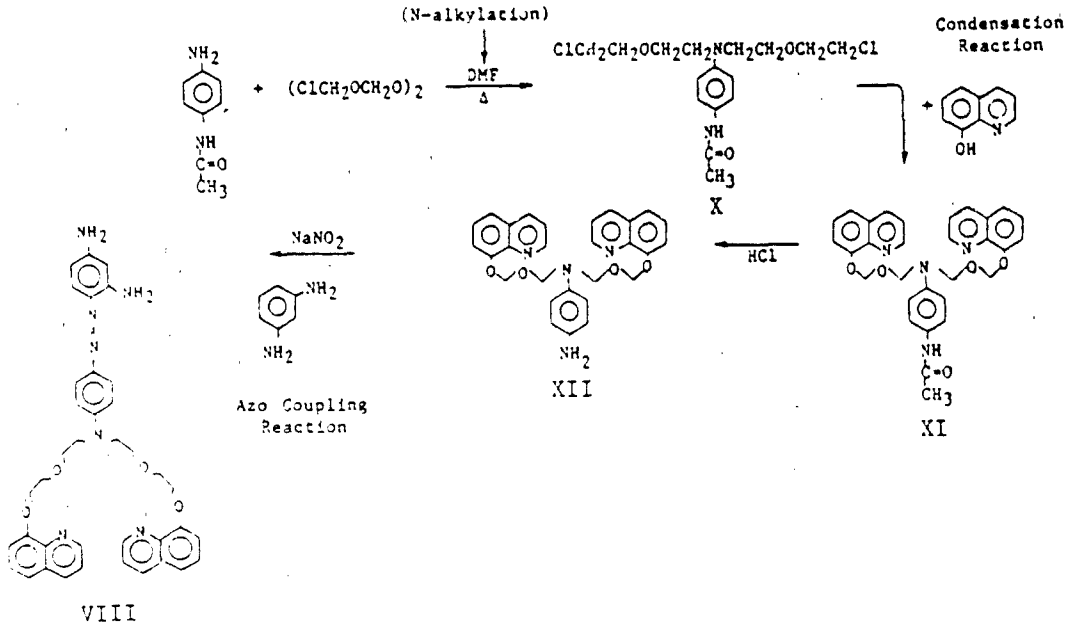


Figure 3. Summary of synthetic steps for preparation of quinoline based chromagenic host.

ii) Syntheses of acyclic polyether chromaphore containing triphenylphosphine oxide endgroups (IX).

The general sequence of reaction steps pursued here are summarized in Figure 4 below.

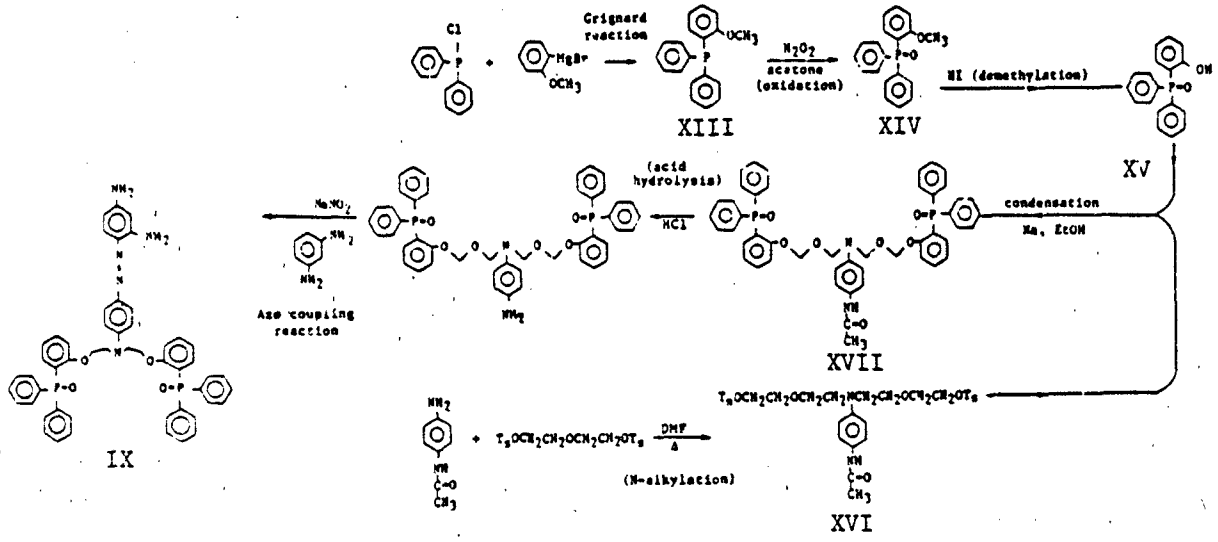


Figure 4. Summary of synthetic steps for preparation of phosphine oxide based chromagenic host.

Magnesium (1.5g) was initially added to a THF solution (30ml) of 1-bromo-2-methoxybenzene (10g) and stirred to initiate the Grignard reaction. After reaction, excess Mg was filtered and the solution cooled to -30°C. To this solution was added 11g of diphenylchlorophosphine in 20ml of THF. After stirring for 4 hours the reaction mixture was warmed to room temperature and stirred overnight before adding saturated potassium carbonate solution to the mixture. The liquid layers were separated using a separation funnel and the organic layer dried overnight using saturated $CaSO_4$ prior to filtration and removal of THF to give (o-methoxyphenyl)diphenylphosphine (XIII) in 75% yield (11.6g). The resulting (o-methoxyphenyl)diphenylphosphine was then oxidized with 30% H_2O_2 (5g) in acetone giving 11.0g of the phosphine oxide (XIV). The methoxy group was now replaced with hydroxy by heating in chloroform with 5g of HI for 2-3 hours to give compound (XV).

The desired amino acid host (IX) was prepared using the same method as for (VIII), except that 40g of bis(2-tosylethylether) was used instead of bis(2-chloroethylether). Here compound (XVI) was added to a THF solution of o-hydroxylphenyldiphenylphosphine oxide (XV) to which had been added an equimolar concentration of sodium ethoxide to catalyze the reaction. This mixture was then heated at 100°C for 6 hours, cooled and worked up as previously described for compound (XI) to give (XVII) in 38% yield. Subsequent steps to give compound (IX) from (XVII) were carried out using procedures previously discussed in the synthesis of (VIII).

Acyclic polyether chromagenic hosts (VIII) and (IX) synthesized during this task were now evaluated as candidate hosts for the reversible detection of amino acids when immobilized onto fiber optic waveguides.

B. Determination of Wavelength Absorption Maxima for (VIII) and (IX) and Wavelength Shifts for VIII-Amine/Amino Acid and IX-Amine/Amino Acid Complexes.

The objective here was to determine wavelength maxima for host compounds (VIII) and (IX) and corresponding wavelength shifts when exposed to amino acid containing solutions. Here cellulose acetate (0.2g) was dissolved in 20ml of acetone along with 0.003g of either the quinoline or triphenylphosphine oxide containing chromagenic hosts. These solutions were initially sprayed onto a silica glass slide using an airbrush and permitted to dry. The spray coated slides were then placed into a cuvette containing a solution of 0.1N $HClO_4$. Spectra were obtained using a Bausch and Lomb Spectronic 21 between the wavelengths 350-950nm. This solution was then replaced with 0.1N $HClO_4$ containing 1g/200ml phenylalanine and allowed to sit for 20 minutes prior to acquiring the spectra again. Corresponding absorbance vs wavelength plots found for respectively chromagenic hosts (VIII) and (IX) are shown in Figures 5 and 6.

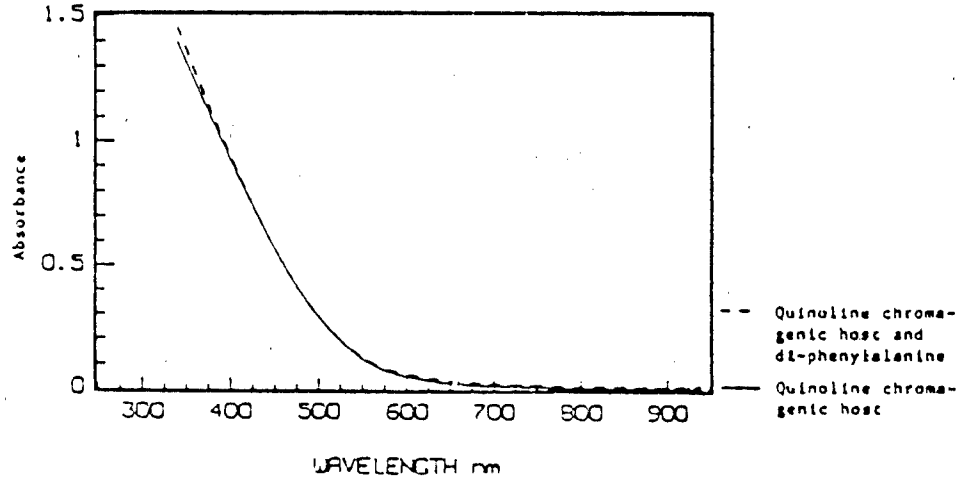


Figure 5. Comparison of absorbance vs wavelength plots for immobilized quinoline chromagenic host with and without phenylalanine.

As can be seen, no major differences in spectra between host and host/guest complexes were apparent. In any event, the depth of penetration by the evanescent wave at the fiber optic/host interfacial region (Figure 2) is dependent, in part, upon relative refractive indices between the fiber optic (n_1) and that of the immobilized chromagenic host or host/guest complex (n_2). Hence, perturbation in n_1/n_2 as a consequence of host/guest binding between the subject acyclic polyethers and a given amino acid might be expected to be detectable by changes in n_2. Furthermore as is well documented, absorbance changes with respect to concentration for a given species of interest are given by the Lambert-Beer law from:

$$A = \varepsilon bc \qquad (4)$$

where A corresponds to absorbance, ε the molar absorptivity, b the light path length through the medium containing species of interest, and c is the species

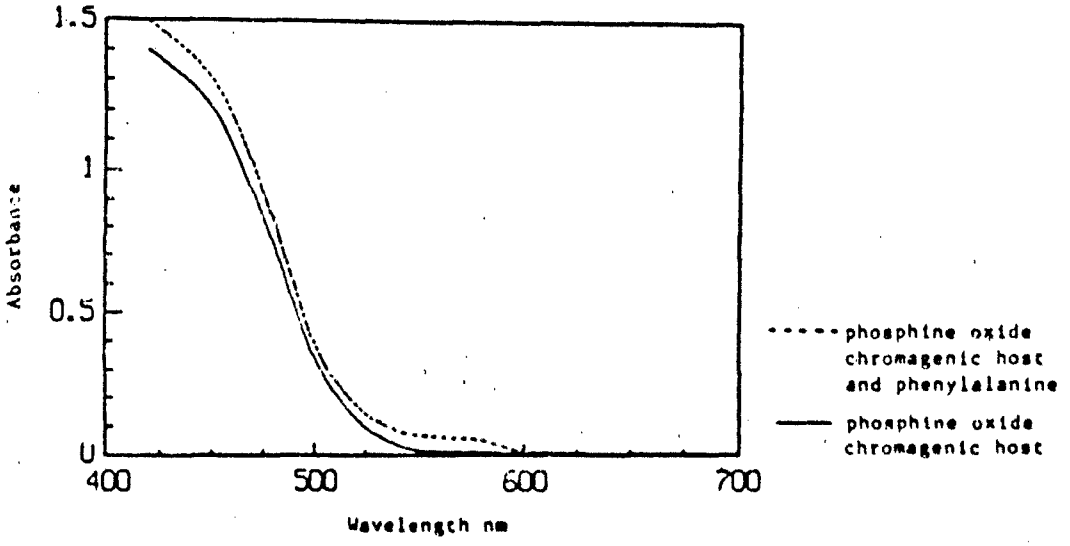


Figure 6. Comparison of absorbance vs wavelength plots for immobilized phosphine oxide chromagenic host with and without phenylalanine.

concentration. Thus a species concentration change will be expected to give, at a given wavelength, a directly related change in evanescent wave absorbance for light transmitted through the optical fiber and result in a consequent modulation in the light intensity. However, since the molar absorptivity A is dependent upon the refractive index of the medium, which in the case of interest here will in-part be dependent upon interaction of the evanescent wave at the host, guest/fiber optic waveguide interface, then incorporating refractive index n_2 into (4) above will give:

$$A = \frac{\varepsilon n_2}{(n_2^2 + 2)^2} \cdot bc \qquad (5)$$

Therefore, from the above, changes in refractive index can be expected to modify transmitted light intensity through an optical fiber even if little or no change in absorption wavelength shift occurs. Additionally, inclusion of an amino acid guest into the chromagenic host cavities could be expected to result in modification of the host dielectric properties, thus also influencing the evanescent wave and leading to a signal change associated with amino acid binding.

As will become evident later, such optical changes did unequivocally result in our ability to reversibly detect amino acids using the subject immobilized chromagenic hosts. Furthermore, optical changes detected were found to bear a linear relation to the concentration of amino acid (phenylalanine) introduced into the fiber optic detector cell for a given residence time and solution flow rate.

Hence optical detection of host/guest chemistry at a fiber optic evanescence wave sensor, in work reported here, did not appear to exclusively rely upon distinct wavelength dependent absorbance changes being present but in part upon interaction between the evanescent wave and changes in refractive index or dielectric properties associated with host/guest chemistry at the interfacial region.

C. Fabrication and Evaluation of (VIII) and (IX) in Cellulose Acetate Membranes Immobilized on Fiber Optic Probes for Amine and Amino Acid Detection.

The objective here was to determine the utility of immobilized chromagenic hosts (VIII) and (IX) present on the outside of an unclad optical fiber for the reversible optical detection of amino acids in aqueous solution. Amino acids of immediate interest in this Phase I effort included phenylalanine, dopa (3-hydroxytyrosine) and tryptophan.

We will now discuss electronics used for fiber optic instrumentation, optic fiber coating procedures and results obtained for the detection of amino acids in aqueous solutions.

i) Electronic circuitry for fiber optic sensor.

As shown schematically in Figure 7, the transmitter circuit used was based around a 555 timer arranged in a basic astable circuit with an output of about 14kHz (2/3 on, 1/3 off). The output was connected to a D44HS power transistor used to drive the super bright LED. The LED, which has a peak emission at 660nm with a luminous intensity of 5000mcd, was coupled to a 1000um fiber optic cable leading to the optical sensor and reference circuits.

The receiver was designed around two similar independent circuits, the first being the signal channel and the second the reference channel. Here light from sensor and reference cables was coupled into two Sharp BS530UV photodiodes. As seen from Figure 8, this detector had a very wide range of sensitivity 200-1150nm with 500-900nm falling above 80% peak sensitivity. The use of signal and reference channels along with the use of sample and hold circuitry was chosen to provide low background drift and high signal to noise capability. The overall principles behind the circuit were based upon a synchronous sample-and-hold circuit first developed at Sandia National Laboratories.[12]

The photodiode was connected to the MOS/FET inputs of a CA3140A op. amp. The output was then fed through a DC blocking capacitor into an OP07 op. amp. circuit to further amplify the signal which was then fed into a SMP81 sample and hold (S/H) circuit. The S/H signal was controlled by the 555 timer chip permitting synchronous detection of small optical changes. The sample and hold output was fed into a RC filter to remove any transients. In the final stage the signal was amplified to give a voltage directly proportional to the light coming from the sensor.

The use of a reference signal channel compensated for the well known drift in LED output with temperature. As a consequence the signal channel was subtracted from the reference channel thereby producing a stable baseline signal.

In addition to the receiver/transmitter circuits, a digital data acquisition system (Figure 9) was fabricated to acquire and process the sensor signal using software control. This data acquisition system consisted of an analog to digital converter, memory and microprocessor. The microprocessor used was an Intel 80C52AH BASIC with built-in BASIC computer language, and an EPROM programmer, directly attached to a PC for data transfer.

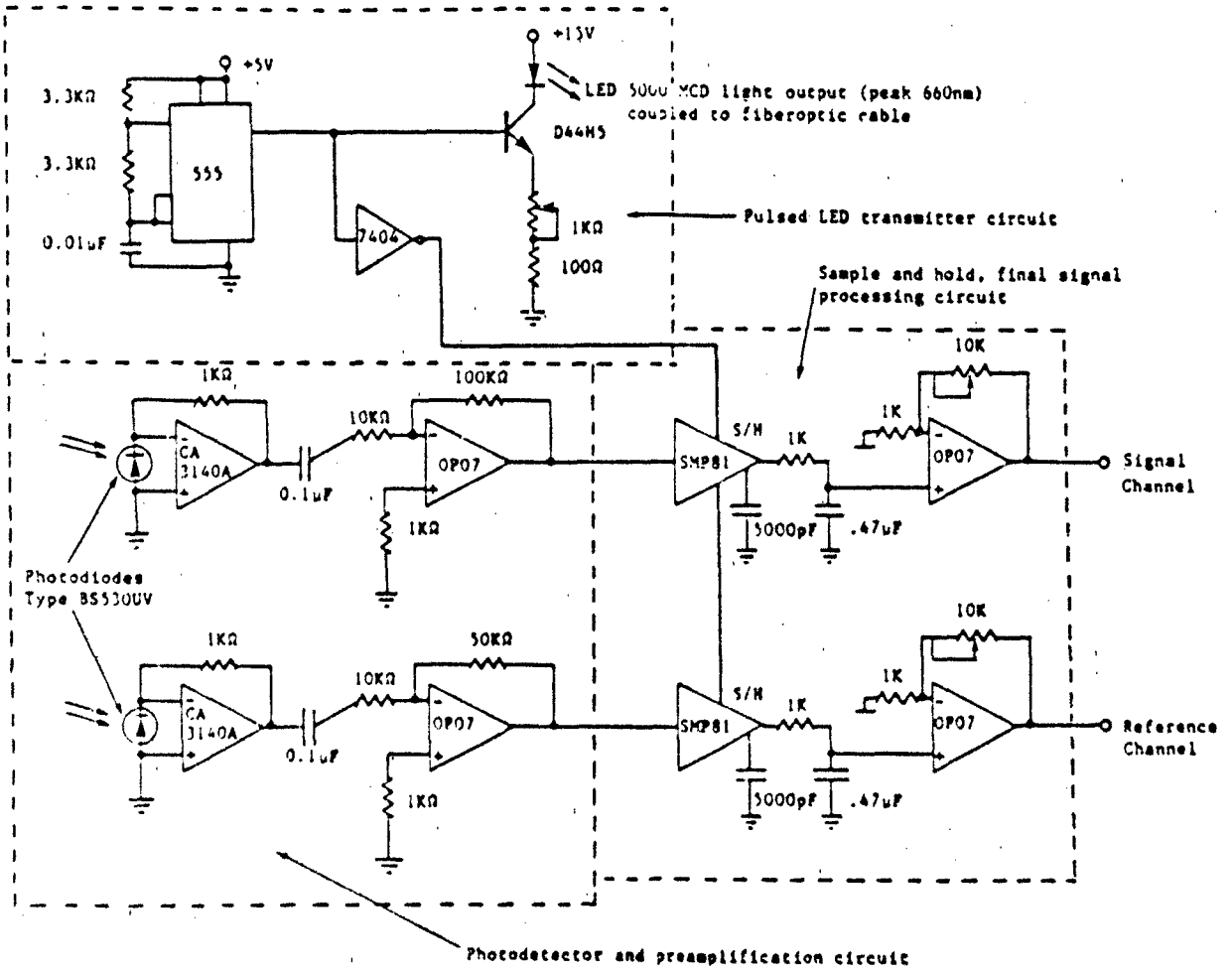


Figure 7. LED/Photodetector circuit for optical waveguide sensor.

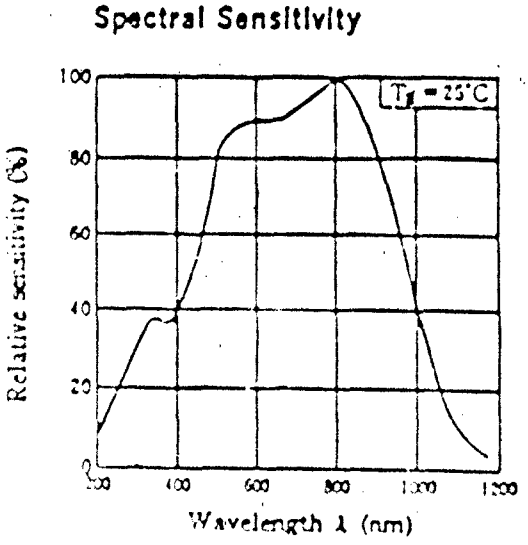


Figure 8. Spectral sensitivity of the BS530UV photodiode used for detecting optical signals in this work.

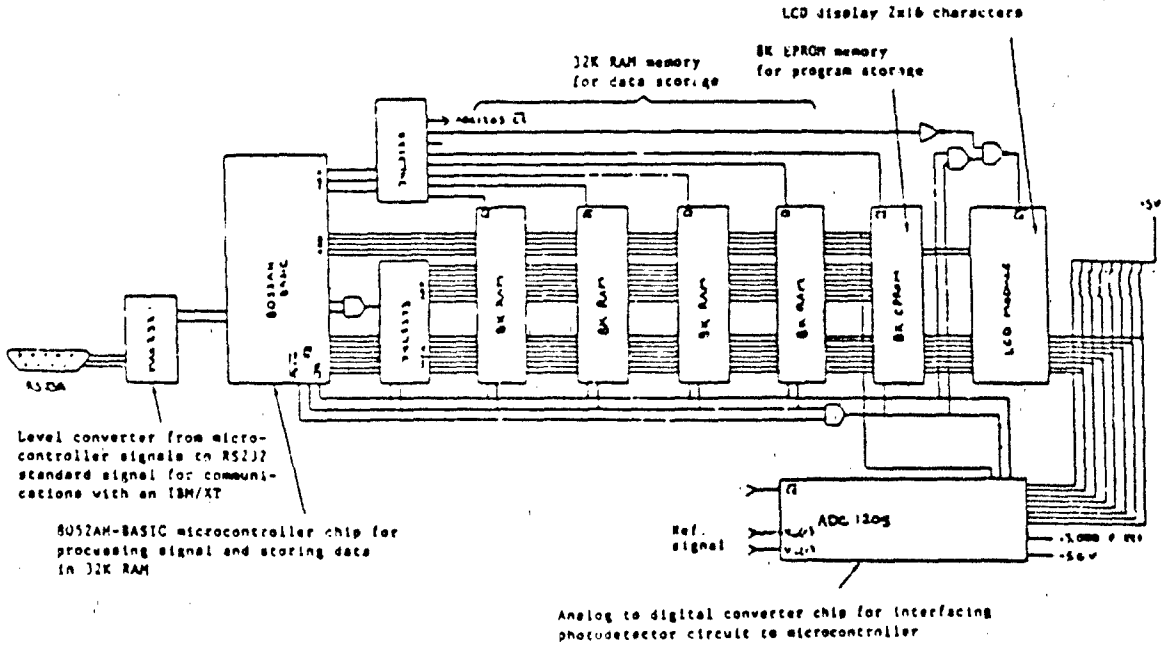


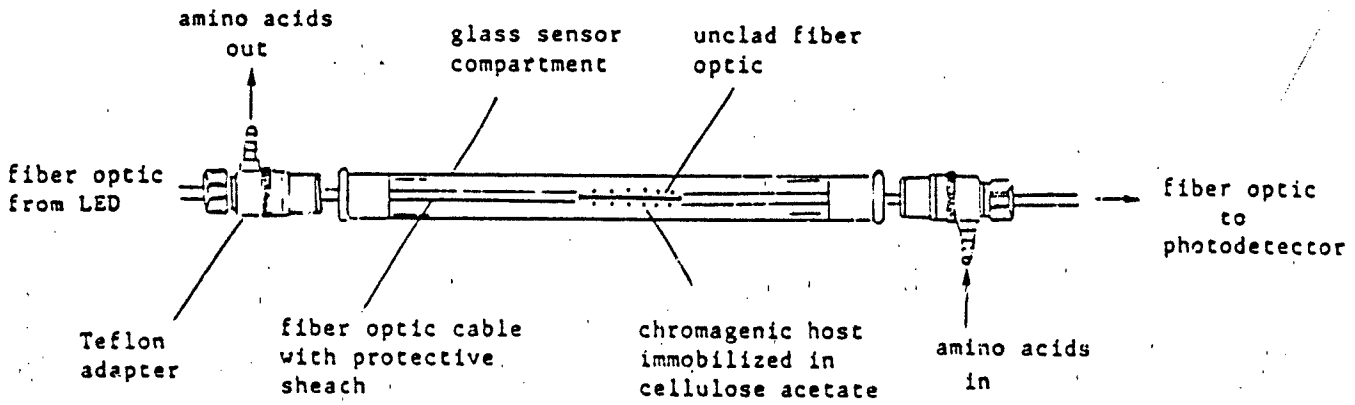
Figure 9. AD/DA converter circuitry for digital data acquisition.

Analog to digital conversion was performed using a National Semiconductor ADC1205CCJ chip which had a resolution of 12 bits plus sign and a conversion time of 100us. Depending on the voltage window being sampled, resolution ranges from 1.2 to .12mV were achievable. Finally, the microprocessor was wired to an alpha-numeric LCD module to monitor the photovoltage and other relevant information.

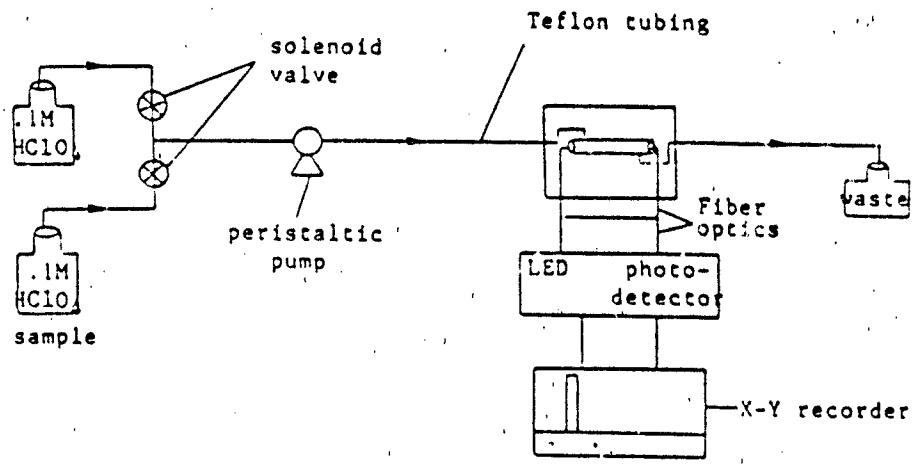
Initial operation of the prototype consisted of zeroing the signal and reference channels while the optical sensor was "turned off". Once zeroed, the unknown amino acid guest molecule dissolved in aqueous solution was introduced into the glass compartment of the fiber optic sensor (Figure 10a) via a peristaltic pump and the signal channel adjusted for signal optimization. This was then stored in the data acquisition system for subsequent graphic output.

ii) Preparation of fiber optic sensor.

The fiber optic cable used here possessed a 1000μm polymethylmethacrylate core. Generally a 2 inch section of the fiber optic cable jacket was initially carefully removed using a sharp blade. This was followed by highly abrading with 220 grit fine alumina sandpaper for cladding removal. The chromagenic host was either 0.05 or 0.02g/20ml and the cellulose acetate 0.05g/20ml in the acetone solvent. This solution was sprayed onto the fiber optic using an air brush. A few passes were made along the length of the exposed fiber and the solvent allowed to evaporate prior to another deposit being made. This was repeated until 10ml of the coating solution had been used. The coated fiber was allowed to dry for 10-30 minutes and then mounted in the glass sensor cell compartment (3cm long, 2mm OD), shown in Figure 10a. A schematic of the overall experimental arrangement used for amino acid optical detection is shown in Figure 10b. Operation of the sensor involved initially filling the glass sensor compartment with aqueous solution (0.1N $HClO_4$) using a peristaltic pump prior to turning on the sensor circuitry. The solution was



a) Fiber optic cell arrangement.



b) Overall experimental arrangement for introduction and removal of amino acid containing aqueous solution.

Figure 10. Experimental arrangement used for optical detection of amino acids.

then passed through the cell at a flow rate of 12ml/min^{-1} to provide a background signal. Flowing solutions were now changed for those containing dissolved amino acids of interest for detection while the voltage output from the photodetector was monitored. Photographs of the actual fiber optic cell and overall experimental arrangement used during this Phase I program are shown respectively in Figures 11 and 12.

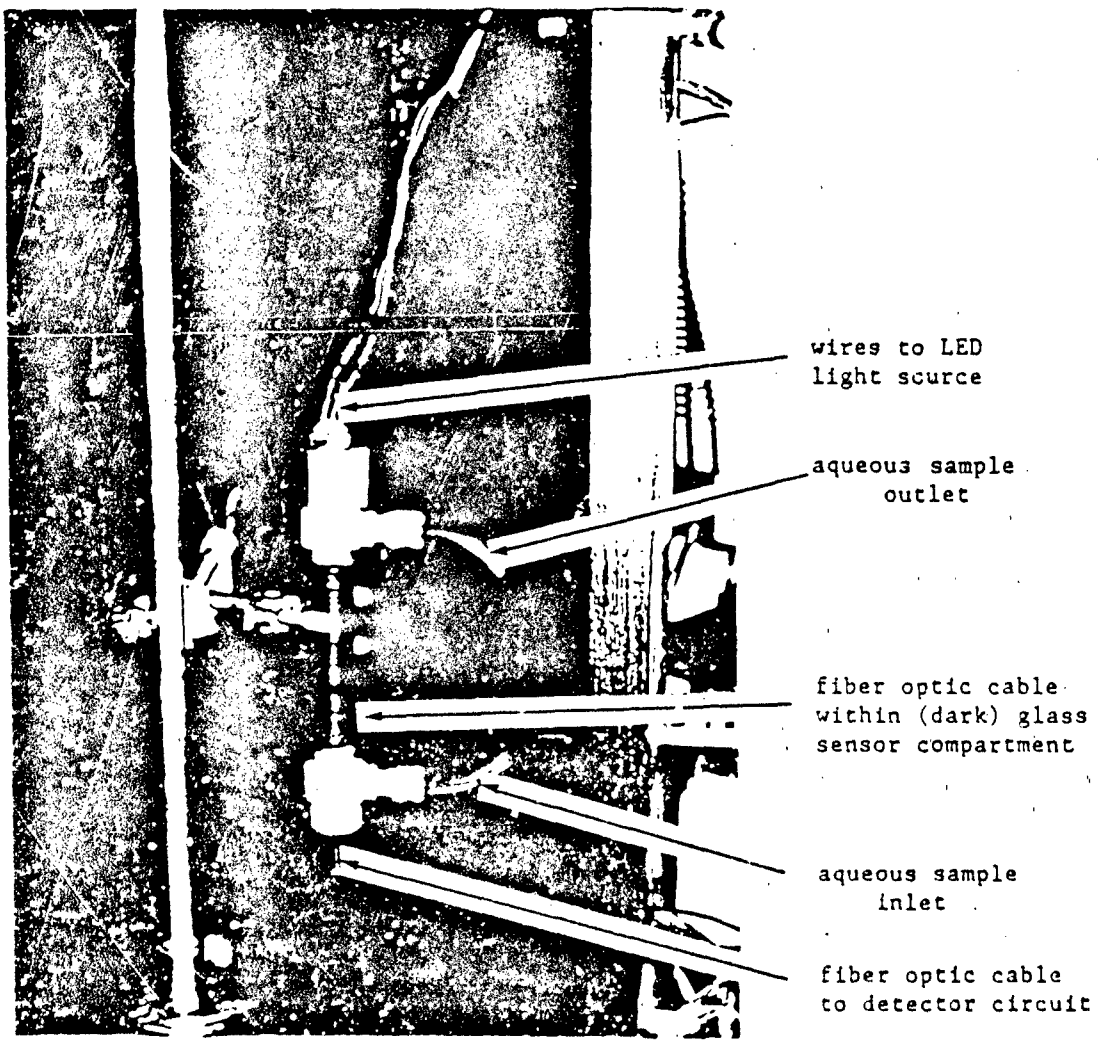


Figure 11. Photograph of fiber optic cell arrangement used during this investigation for reversibly detecting amino acids in aqueous solution.

iii) Fiber optic sensor evaluation for the reversible detection of amino acids in aqueous solution.

All measurements were performed with appropriate experimental controls being taken which included a) initially passing aqueous solution, with and without the amino acid of interest, past the exposed fiber optic in the absence of any surface coating, b) repeating the above experimental sequence with a sprayed cellulose acetate film but in the absence of an incorporated chromagenic host, and c) again repeating the above experimental sequence with the chromagenic host incorporated within the cellulose acetate coating on the fiber optic surface. In all cases during this work no detectable optical

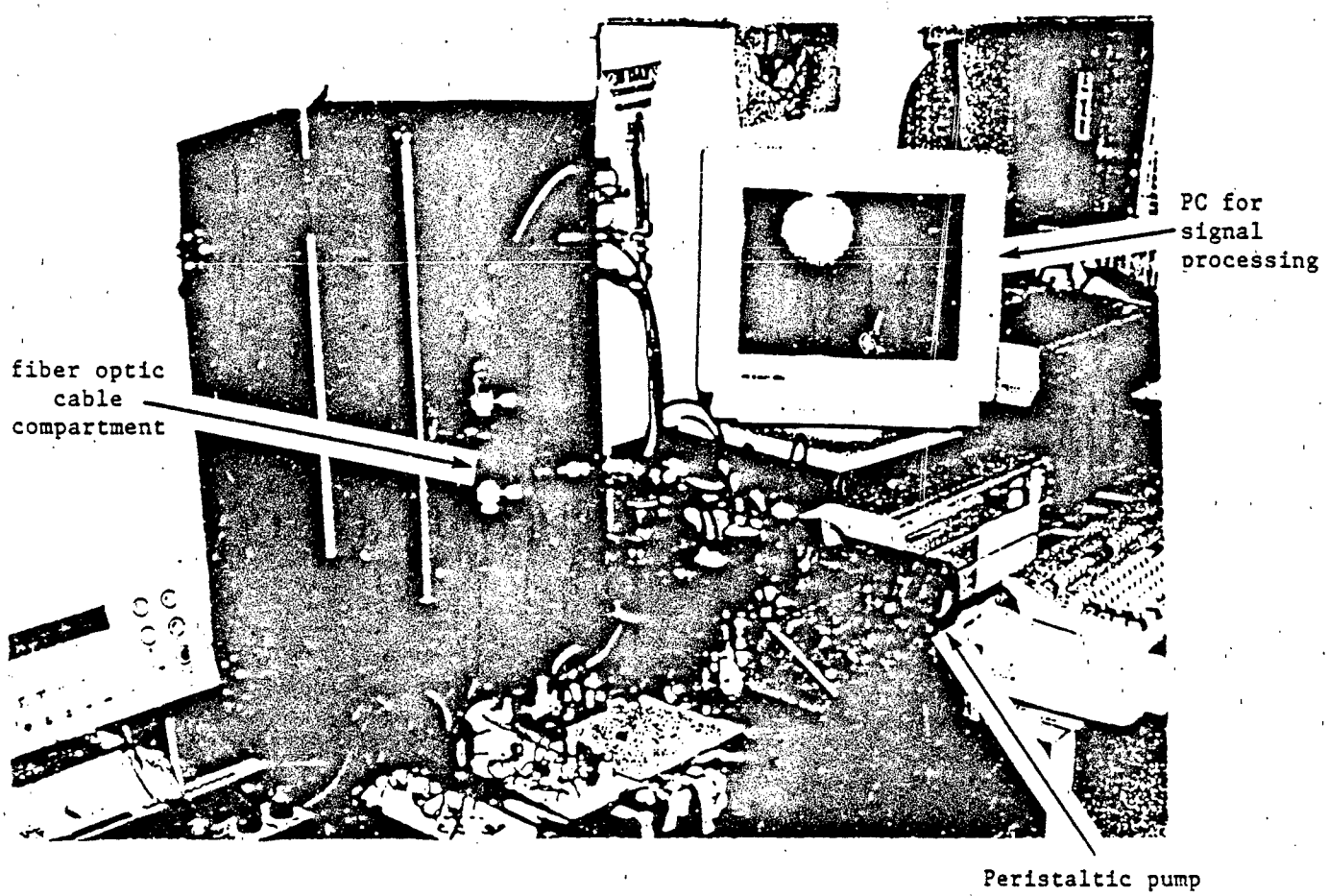


Figure 12. Photograph of overall experimental arrangement used in this Phase I program for optically detecting amino acids in aqueous solution.

response was found in the absence of the subject chromagenic hosts incorporated within the cellulose acetate coating on the fiber optic.

Figure 13 shows typical results for a fiber optic coated with the chromagenic host (VIII) to 1g of phenylalanine in 200ml of 0.1N $HClO_4$. The fiber optic was coated using the previously discussed procedure. Upon passing the amino acid containing solution past the fiber optic, a distinct photovoltage absorption signal was observed corresponding to perturbation of the evanescence signal within the fiber optic. Upon replacing the amino acid containing $HClO_4$ with a solution containing only $HClO_4$, the optical signal was removed. The observed response time was dictated by the solution flow rate into the sampling fiber optic cell. Most significant was the observation that these signals were 100% reversible.

As one might expect, incorporating a higher concentration of the chromagenic host within the cellulose acetate film on the fiber optic might result in

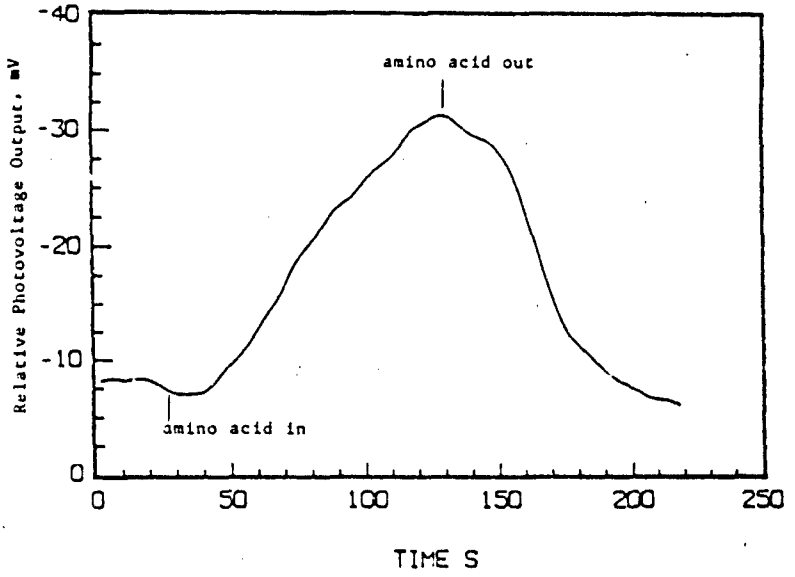


Figure 13. Phenylalanine response at evanescent wave optical sensor. Solution = 1g phenylalanine in 200ml H_2O (0.1N $HClO_4$). pH = 2-3, fiber was spray coated with 20ml of solution containing .005g of quinoline chromagenic host and 0.05g cellulose acetate. Solution flow rate 12ml/min.

signal enhancement. Tnis expectation can be seen in Figure 14 where a four times higher concentration (0.02g) of the chromagenic host (VIII) was used in the acetone/cellulose acetate solution sprayed onto the fiber optic waveguide and the corresponding photovoltage output for the same concentration of phenylalanine was found to approximately double.

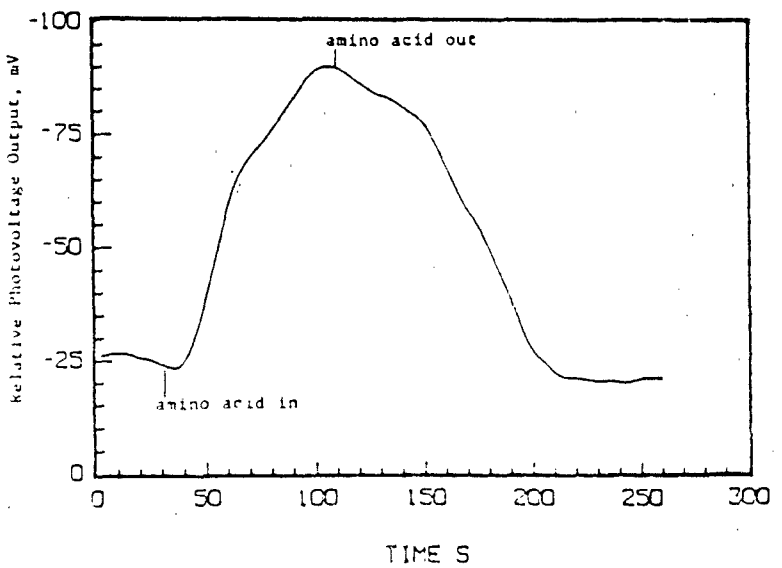


Figure 14. Phenylalanine response at evanescent wave optical sensor. Solution = 1g phenylalanine in 200ml H_2O (0.1N $HClO_4$). pH = 2-3, fiber was spray coated with 20ml of solution containing .02g of quinoline chromagenic host and 0.05g cellulose acetate. Solution flow rate 12ml/min.

This gives further evidence that the optical change observed is dependent upon the chromagenic host and may be explained by an association of the type represented previously in Figure 1.

Using the same coated fiber optic as in Figure 14, we evaluated the response of immobilized host (VIII) to the amino acid tryptophan. Interestingly, no detectable optical response was found compared to phenylalanine. This experiment was now repeated using the following experimental sequence for passing aqueous solutions through the fiber optic containing cell: 1) aqueous solution (0.1N $HClO_4$) containing no amino acid, 2) aqueous solution containing tryptophan, and lastly 3) aqueous solution containing both tryptophan and phenylalanine. The resulting optical response curves obtained from this sequence of experiments are summarized in Figure 15.

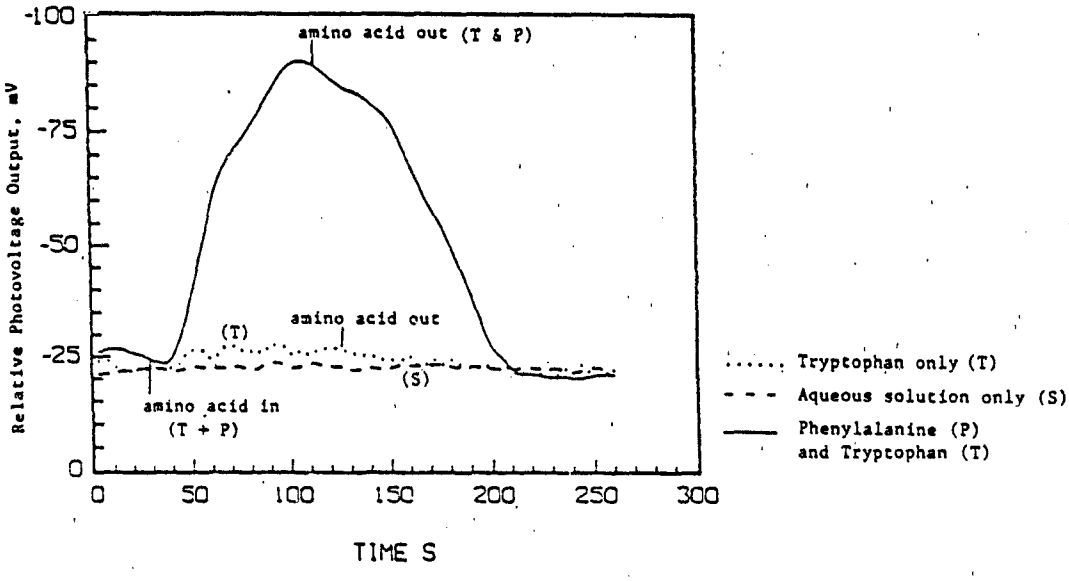
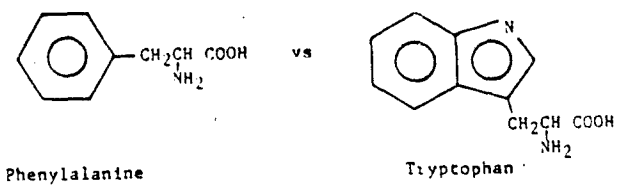


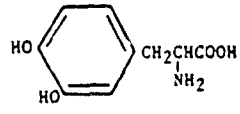
Figure 15. Comparison of optical waveguide sensor response to a) aqueous 0.1N $HClO_4$, b) tryptophan (1g/200ml), and c) phenylalanine (1g/200ml + tryptophan (1g/200ml) at an evanescent wave optical sensor coated with quinoline chromagenic host (VIII). Solution flow rate 12ml/min.

The significant experimental observation here was that no optically detectable response was evident upon exposing the quinoline based host (VIII) to tryptophan and that the corresponding signal for phenylalanine when using tryptophan/phenylalanine mixtures was also identical to that for phenylalanine solutions where tryptophan was absent (Figure 14). Thus, the chromagenic host (VIII) shows selectivity towards phenylalanine in the presence of tryptophan. The reason for this selectivity may be a consequence of steric differences between phenylalanine and tryptophan associated with their interaction with the immobilized host (VIII):



Phenylalanine Tryptophan

As has been previously discussed, the incorporation of hydroxy groups onto the aromatic ring of a given amino acid would be expected to decrease cation hydrophobicity, thus resulting in somewhat weaker binding to the acyclic polyether host and correspondingly smaller optical response. To examine this further we investigated the optical response from 3-hydroxytyrosine (dopa) corresponding to the addition of two hydroxy groups to phenylalanine.



3-hydroxytyrosine (dopa)

The corresponding optical response curves for two loadings of fiber optic supported chromagenic host (VIII) to 3-hydroxytyrosine are summarized in Figures 16 and 17.

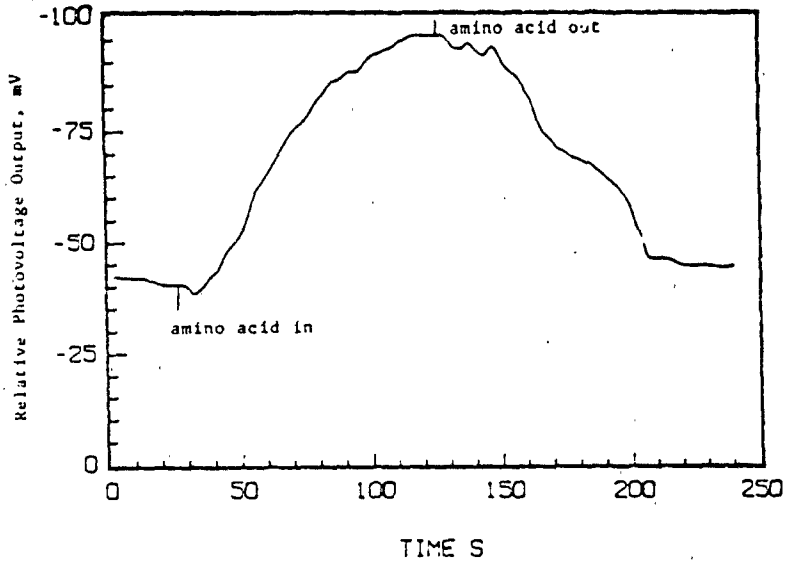


Figure 16. 3-hydroxytyrosine (dopa) response at an evanescent wave optical sensor. Solution = 1g/200ml H_2O (0.1N $HClO_4$). pH = 2-3. Fiber was spray coated with 20ml of solution containing .005g quinoline chromagenic host (VIII) and 0.05g cellulose acetate. Solution flow rate 12ml/min^{-1}.

Again, as previously shown in Figures 14 and 15 for phenylalanine, higher apparent loadings of the chromagenic host on the fiber optic resulted in enhanced optical signals. The actual loadings of chromagenic host (VIII) deposited on fiber optics from which optical responses were obtained were not known, however, with great accuracy at this stage.

Our general observation was, however, that for immobilized chromagenic host (VIII) supported on fiber optic sensors in nominally the same manner, somewhat smaller optical signals were found for 3-hydroxytyrosine compared to phenylalanine reflecting a weaker binding by 3-hydroxytyrosine to the acyclic polyether host cavity. In any event, the optical response obtained from the fiber optic supported chromagenic host, when exposed to aqueous solution containing this amino acid, was found to be totally reversible.

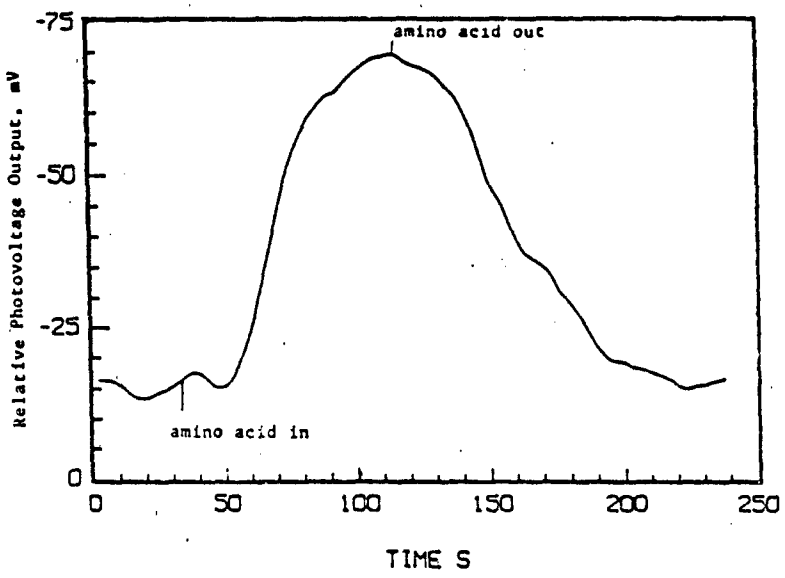


Figure 17. 3-hydroxytyrosine (dopa) response at an evanescent wave optical sensor. Solution = 1g/200ml H_2O (0.1N $HClO_4$). pH = 2-3. Fiber was spray coated with 20ml of solution containing .02g quinoline chromagenic host and 0.05 cellulose acetate. Solution flow rate 10ml/min.

Fiber optic sensors incorporating the immobilized phosphine oxide chromagenic host (IX) were also evaluated for the optical detection of phenylalanine. Here although good sensor response and reversibility was observed, a significant baseline drift was evident as shown in Figure 18. Consequently emphasis was placed on the quinoline chromagenic host (VIII).

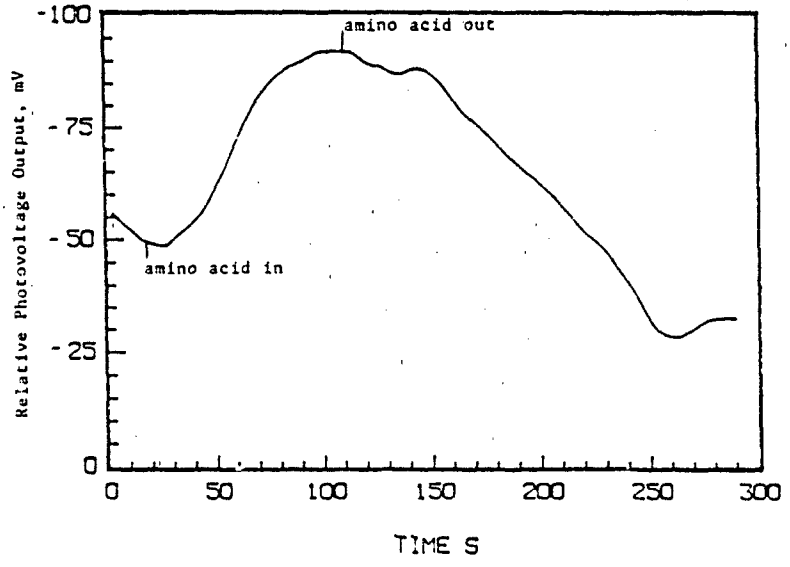


Figure 18. Phenylalanine response at an evanescent wave optical biosensor. Solution = 1g phenylalanine in 200ml H_2O (0.1N $HClO_4$). pH = 2-3. Fiber was spray coated with 20ml of solution containing .005g phosphine oxide chromagenic host and 0.05g cellulose acetate. Solution flow rate 12ml/min.

We now investigated whether there was any clear correlation between the fiber optic response and the concentration of amino acid present in the solution passed through the fiber optic glass sensor compartment. This was performed using the quinoline chromagenic host (VIII) deposited onto the fiber optic waveguide as previously discussed (0.02g/20ml acetone and 0.05g cellulose acetate/20ml acetone). Into the glass fiber optic sensor compartment was passed phenylalanine in 0.1N $HClO_4$ between the respective concentrations 136 and 544ppm/mole. Solution flow rate was 12ml/min. Figure 19 summarizes the photovoltage output obtained from the fiber optic for four phenylalanine concentrations. As is evident, a clear correlation was observed between phenylalanine concentration and the detected optical response. In these measurements the phenylalanine containing solution was passed through the cell for between 100 and 113 seconds after which only 0.1N $HClO_4$ was passed. Upon plotting the photovoltage output response obtained after passing the phenylalanine solution through the detector for 100 seconds versus the initial phenylalanine concentration, we can see that a reasonably good linear correlation could be obtained as shown in Figure 20.

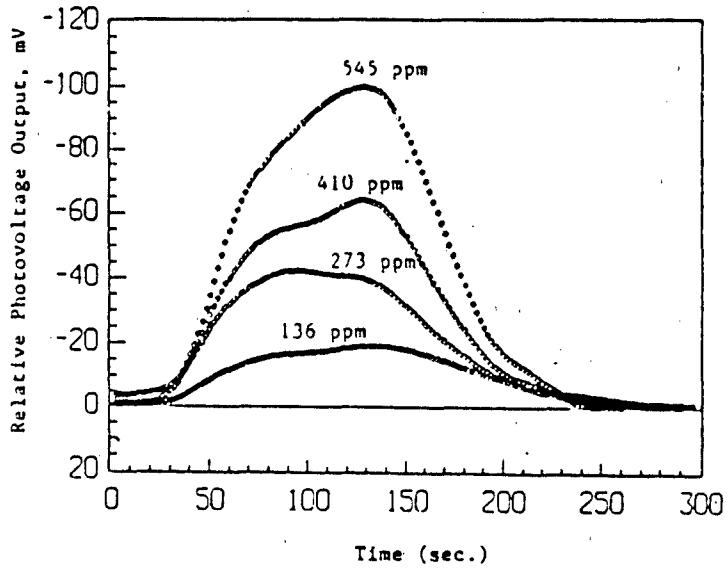


Figure 19. Sensor response to four different phenylalanine concentrations (ppm values are mole based) at a quinoline host modified poly(methylmethacylate) fiber optic sensor.

In summary, the above discussed results are all in support of acyclic polyether hosts, when immobilized onto fiber optic substrates, for achieving reversible, selective and quantitative detection of amino acids in aqueous solution. As we have previously discussed, optical detection of the subject host/guest chemistry, after interaction with the evanescent wave, did not appear to exclusively rely upon distinct wavelength dependent absorbance changes being present but possibly upon perturbations in refractive index or dielectric properties at this interfacial region. Increased selectivity and sensitivity for a given host/guest association might be expected to be enhanced further,

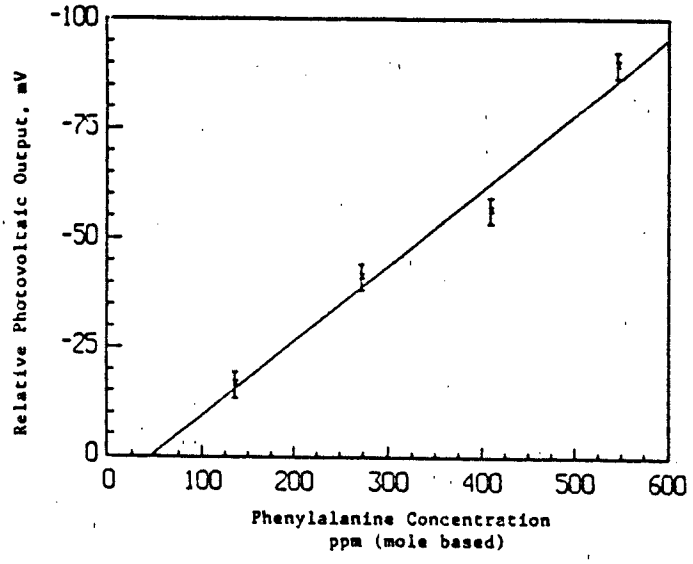


Figure 20. Relationship of relative photovoltaic output of quinoline host modified fiber optic sensor to phenylalanine concentration after 100 seconds solution flow through detector.

however, by the judicious selection and incorporation of chromagenic groups within immobilized host molecules on the fiber optic substrate which produced distinct absorbance signals. Furthermore, we anticipate that the response and sensitivity of the subject evanescent wave fiber optic detector may also be improved by i) increasing the length of the fiber optic/host:guest interaction region, and ii) increasing both the LED output and photodetector gain. We anticipate that this will permit detection levels for phenylalanine and 3-hydroxytyrosine to be achieved in the ppb/mole (0.001g/200ml) region using the current approach.

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